United States Department of Agriculture
Grain Inspection, Packers and Stockyards Administration
Federal Grain Inspection Service

FGIS Issuance Change

CHANGE TO

9 DIRECTIVE **9** MANUAL X**9** HANDBOOK

CHANGE NO:	**TO** *(No.)*	**TITLE:**	**DATE:**
2		Don (Vomitoxin) Handbook	12-23-02

PURPOSE OF CHANGE: The Don (Vomitoxin) Handbook has been revised to add the Vicam - DON FQ test to the list of FGIS approved test kits and to include a new chapter dedicated to the test procedures. Other revisions include modification to the testing procedures for the Veratox 5/5 test kit, the expanded use of microwell readers for the RIDASCREEN® FAST DON test kit, and the elimination of "estimated" test results.

FILING INSTRUCTIONS

Insert Chapter 12, dated 12-23-02, into the handbook and make the following page changes.

Remove	Dated	Insert	Dated
Table of Contents	12-17-01	Table of Contents	12-23-02
Page 1-1	12-17-01	Page 1-1	12-17-01
Page 1-2	no date	Page 1-2	12-23-02
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Chapter 11	12-17-01	Chapter 11	12-23-02

Retain this issuance sheet as an aid in verifying handbook contents.

/s/ David Orr

David Orr, Director
Field Management Division

Distribution: A, C, E Originating Office: PPB, FMD

U.S. DEPARTMENT OF AGRICULTURE
GRAIN INSPECTION, PACKERS AND STOCKYARDS ADMINISTRATION
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CHAPTER 12

VICAM - DON FQ TEST KIT

12.1 TESTING AREA

The extraction solution and other materials used in the DON FQ test kit necessitates the use of separate FGIS-approved laboratory space. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this handbook to ensure a safe and efficient work environment.

12.2 EXTRACTION PROCEDURES

a. Preparation of Extraction Solvent (84 Percent Acetonitrile Solution).

Make up the solution by using the ratio of 84 parts acetonitrile to 16 parts deionized/distilled water. Prepare the 84 percent acetonitrile solution by adding 840 ml acetonitrile to 160 ml of distilled or deionized water. Mix well. Label the solution bottle and keep it tightly capped when not in use.

If the amount of solution being prepared needs to be adjusted based on the workload at individual locations, make sure that 84 parts acetonitrile to 16 parts deionized/distilled water ratio is maintained.

b. Extraction Procedures.

(1) Place 50 grams of ground sample into a blender jar.

(2) Add 200 ml of acetonitrile/water (84/16) and blend on high for 3 minutes.

(3) Filter into a sample container using Vicam fluted filter paper, Whatman No. 1 filter paper, or equivalent.

12.3 TEST PROCEDURES

a. Purification Procedures.

NOTE: All solution transfers may be carried out using adjustable automatic pipettors with disposable tips. Care should be taken to make sure that the tips used are large enough to hold the volume being transferred. Make sure that they are securely attached to the pipettor.

(1) Place 4 ml of extract in a 15 x 85 culture tube. Insert a DON FQ column into the top of the culture tube and slowly (20 seconds) push to the bottom of the tube.

(Note: Use 6 ml of extract and a DON FQ MB column for malted barley samples and take 30 seconds to push the extract through the column.)

(2) Transfer 1.5 ml of each purified sample extract to a 12 x 75 mm cuvette. Use a clean pipette tip for each transfer.

b. Calibrators and Control Preparation.

(1) Allow the calibrator and control solutions to come to room temperature.

(2) Invert each calibrator standard bottle and control standard bottle three times to mix thoroughly.

(3) Transfer 1.5 ml of the green-labeled calibrator solution to a clean 12 x 75 mm cuvette.

(4) Using a clean tip, transfer 1.5 ml of the red-labeled calibrator solution to a clean 12 x 75 mm cuvette.

(5) Using a clean tip, transfer 1.5 ml of the control (yellow-labeled) solution to a clean 12 x 75 mm cuvette.

(6) Cap the calibrator solutions tightly and store in the refrigerator.

(7) Proceed with the analysis, treating samples, calibrators, and control identically.

c. Evaporation Procedures.

Evaporate each sample, calibrator, and control to dryness using a vacuum manifold and dry bath set at 70°C.

Note: To decrease the evaporation time, turn off the vacuum to the manifold for the rows that are not being used.

d. Derivatization Procedures.

(1) Add 1.5 ml of Reagent A to all sample tubes, calibrators, and control.

(2) Add 50 microliters (µl) of Reagent B to all sample tubes, calibrators, and control. Cap the tubes and mix contents with a vortex for 10 seconds.

(3) Heat the tubes in a 50°C bath for 10 minutes.

(4) Remove tubes from the bath and cool to room temperature. Read the samples in the fluorometer within 1 hour.

Note: Cuvettes may be placed in tap water for 30 seconds to cool. Dry the outside wall of the cuvette completely before placing in the fluorometer.

e. Fluorometer Reading.

Calibrate the fluorometer using the following procedure:

(1) Turn on the power (no warm-up is necessary).

(2) Change the date or time - If correct, press the "Continue" key.

(3) When asked for Test Delay Time, enter "2" and press the "Enter" key.

(4) When asked for answer format, select "Decimals."

(5) When asked for measurement units, select "ppm."

(6) At the "insert red vial" prompt, place the appropriate calibrator cuvette into the sample well.

(7) When asked for the calibrator value, enter the appropriate value (refer to the card supplied with the calibrators for the red value) and press the "Enter" key.

(8) When asked to "remove the red vial," remove the calibrator tube from the sample well.

(9) At the "insert green vial" prompt, place the appropriate calibrator cuvette into the sample well.

(10) When asked for the "blank value, "enter the appropriate value (refer to the card supplied with the calibrators for the green value) and press the "Enter" key.

(11) When asked to "remove the green vial," remove the calibrator tube from the sample well.

(12) At the "insert test vial" prompt, place the control cuvette into the sample well.

(13) The fluorometer will now display the value for the control vial.

(14) Compare the value of the control with the values listed on the card. If the control value is within the specified range, the fluorometer is ready to analyze samples. If the value is outside of the specified range, rerun the red, green, and yellow calibration cuvettes. If the control value still exceeds the specified range limit, contact Vicam.

(15) Press the "Enter" key. The fluorometer is now ready to analyze samples.

f. Reading the Results.

To determine the DON concentration insert the cuvette containing the sample portion into the sample well of the fluorometer. The DON concentration will appear on the display after the appropriate 2-second delay. Read the results.

12.4 REPORTING AND CERTIFYING TEST RESULTS

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

12.5 SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON result at 9.0 ppm and the conformance limit value is 5 ppm, in order to obtain a true value, dilute 5 ml of the original extract with 10 ml of the extraction solution (acetonitrile/water). The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 3.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

The calculation is as follows:

$$\text{True DON Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result}$$

In this example: True DON Value = (15 ÷ 5) x 3.1 ppm
= 3 x 3.1 ppm = 9.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the conformance limit of the test kit.

12.6 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

12.7 WASTE DISPOSAL

Transfer sample extract solutions (acetonitrile/water) and derivatization solutions into a liquid waste container for disposal. Follow SOP, established by the field office, for handling and disposing of hazardous waste.

Transfer sample slurry, used filter paper, cuvettes, caps, and columns into the normal solid waste container for routine disposal.

12.8 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits:

(1) Glass culture tubes (15 x 85 mm) - 25 tubes per test kit

(2) DON FQ (or DON FQ MB for malted barley) columns

(3) Glass cuvettes and caps (12 x 75 mm) - 50 per test kit

(4) Reagent A - Ethylenediamine in methanol

(5) Reagent B - Zirconyl Nitrate in methanol

(6) Calibrator solutions (red-label, and green-label) plus control solution (yellow-label)

b. Materials Required but not Provided:

(1) Blender and blender jars. The unit must be explosion proof.

(2) Funnel

(3) Beakers - 250 ml

(4) 250 ml graduated cylinder

(5) Extraction Solvent - Acetonitrile/distilled or deionized water (84/16)

(6) Filter paper - Vicam part # 31240, Whatman No. 1, or equivalent

(7) Carboy - 2 gallon capacity

(8) Vortex mixer

(9) Pipette and tips - 50 µl

(10) Pipette and tips - 1.5 ml

(11) Fluorometer with printer - Romer RL 100, Vicam Series III and IV, Torbex 100, Vicam FX-100

(12) Vacuum Pump and Trap

(13) E-Vap Evaporator

(14) Test Tube Rack

(15) Thermometer

(16) Dry Bath with Heating Block

(17) Sample grinder

(18) Balance

12.9 STORAGE CONDITIONS

a. Columns.

DON FQ and DON FQ MB columns - Room temperature in a drawer or box.

b. Reagents.

Reagents A & B are shipped in amber bottles, cap tightly and store in a temperature controlled area (between 40° and 80° F). Do not freeze. Reagents should stay stable for 6 months.

c. Calibration and Control Solutions.

Calibration and control solutions are shipped in amber vials, cap tightly and store in the refrigerator. Solutions should stay stable for 6 months.

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CHAPTER 4

CERTIFICATION

4.1 BACKGROUND

Wheat, barley, corn, and oats are tested for DON under the authority of the United States Grain Standards Act (USGSA). Under the USGSA, DON results are recorded on the pan ticket, worksheet, or loading log and in the remarks section of the certificate.

Certify DON test results on grain in accordance with sections 800.160 through 800.166 of the regulations under the USGSA.

Upon the request of the applicant, separate certificates may be issued for grade and for DON when both are determined on the same lot.

Sections 800.125 and 800.135 of the regulations under the USGSA permit a review inspection on either official grade/factors or official criteria. When requested, a review inspection for official grade or official factors and official criteria may be handled separately, even though both sets of results are reported on the same certificate. When official grade or official factors and official criteria are reported on the same certificate, the review inspection certificate shall show a statement indicating that the review results are for official grade, official factors, or official criteria, and that all other results are those of the original, reinspection, or appeal inspection results, whichever is applicable.

4.2 GENERAL PROCEDURES

The type of service requested and the test method used determine how DON results are recorded and certified.

a. Qualitative Testing.

(1) Record the results of a **qualitative service** on the pan ticket and inspection log as being equal to or less than a threshold (e.g., 2 ppm) or as exceeding the threshold.

(2) If a **quantitative method** is used to provide qualitative service, record the test results on the work records in a quantitative measurement (e.g., 1.4 ppm) or a qualitative measurement (e.g., $\leq$ 2 ppm).

(3) Certify results as being equal to or less than a threshold.

b. Quantitative Testing.

(1) Test kits with conformance limit ending in .5 ppm (e.g., 2.5 ppm)

Record the results on the pan ticket and the inspection log to the tenth ppm.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Certify test results that are above the lower conformance level (e.g. 0.6 ppm) and below the upper conformance level (e.g., 2.4 ppm) to the nearest whole ppm. For example: A DON test result of 2.3 ppm obtained using a DON test kit with a conformance range of 0.5 - 2.5 ppm would result in the following certification statement "DON 2 ppm." Upon request of the applicant, the results may be certified to the tenth ppm.

Test results that are equal to the conformance limit are certified as equal to the conformance limit. For example: A DON test result of 2.5 ppm obtained using a DON test kit with a conformance range of 0.5 - 2.5 ppm would result in the following certification statement "DON 2.5 ppm."

Test results greater than the conformance limit are certified as exceeding the conformance limit. For example: A DON test result of 2.8 ppm obtained using a DON test kit with a conformance range of 0.5 - 2.5 ppm would result in the following certification statement "DON exceeds 2.5 ppm."

(2) Test kits with conformance limits rounded to the whole ppm (e.g., 5 ppm)

Record the results on the pan ticket and the inspection log to the tenth ppm.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Certify test results that are between 0.6 ppm and the conformance limit (e.g., 5 ppm) to the nearest whole ppm. For example: A DON test result of 5.4 ppm obtained using a DON test kit with a conformance range of 0.5 - 5 ppm would result in the following certification statement "DON 5 ppm." Upon request of the applicant, results between 0.6 ppm and the conformance limit (e.g., 5 ppm) may be certified to the tenth ppm.

Test results greater than the conformance limit are certified as exceeding the conformance limit. For example: A DON test result of 5.5 ppm obtained using a DON test kit with a conformance range of 0.5 - 5 ppm would result in the following certification statement "DON exceeds 5 ppm."

STANDARD REPORTING - QUANTITATIVE TESTING

Test Kit Range	Test Result	Certify as:	Test Result	Certify as:	Test Result	Certify as:	Test Result	Certify as:
0.5 - 2.5 ppm	0.5 or less	$\leq$ 0.5 ppm	0.6 - 2.4	Nearest whole ppm	2.5 ppm	= 2.5 ppm	2.6 or more	> 2.5 ppm
0.5 - 3 ppm	0.5 or less	$\leq$ 0.5 ppm	0.6 - 3.4	Nearest whole ppm	*	*	3.5 or more	> 3 ppm
0.5 - 5 ppm	0.5 or less	$\leq$ 0.5 ppm	0.6 - 5.4	Nearest whole ppm	*	*	5.5 or more	> 5 ppm

OPTIONAL REPORTING - QUANTITATIVE TESTING										
Test Kit Range		Test Result	Certify as:		Test Result	Certify as:		Test Result	Certify as:		Test Result	Certify as:
0.5 - 2.5 ppm		0.0	Not Detected		0.6 - 2.5	Actual tenth ppm		*	*		*	*
0.5 - 3 ppm		0.0	Not Detected		0.6 - 3.0	Actual tenth ppm		*	*		*	*
0.5 - 5 ppm		0.0	Not Detected		0.6 - 5.0	Actual tenth ppm		*	*		*	*

4.3 CERTIFYING TEST RESULTS

a. Single lot inspection basis for trucks and railcars.

Certify each test result on a separate certificate.

b. Combined land carrier basis for trucks and railcars.

If an applicant requests DON testing on a composite basis (up to 5 railcars and 15 trucks) and the inspection for grade on the basis of individual carriers, factor only certificates are issued for the DON testing and separate grade certificates are issued for each carrier.

c. Composite Sample Testing for Shiplots.

Certify the composite results using the appropriate qualitative or quantitative statements.

d. Submitted Sample Testing.

Certify the results using the appropriate qualitative or quantitative statements.

e. Unit Train and Shiplot Inspection under the CuSum Loading Plan.

(1) Recording Test Results.

DON test results of sublot samples taken throughout loading are recorded on the loading log. A material portion occurs if the sublot result exceeds the limit as specified in the load order.

(2) Certifying Test Results.

When sublot samples are tested using a **quantitative method**, certify the lot based on the mathematical/weighted average (as applicable) of the accepted sublot results. When sublot samples are tested using a **qualitative method**, certify the lot results as equal to or less than the maximum limit (e.g., 2 ppm). If some sublots were reviewed using a quantitative method, continue to certify the lot as equal to or less than the maximum limit.

Certify material portions separately.

(3) Material Portions.

If a material portion occurs, the applicant has the option of requesting a review inspection. Review inspection results replace previous results when determining if a material portion exists.

If a material portion designation due to DON is not removed by the review inspection process, the applicant may leave the material portion on board and receive a separate certificate; return the grain to the elevator; or discharge the material portion along with additional grain in common stowage equivalent to one half the material portion quantity.

4.4 APPROVED STATEMENTS

Upon request of the applicant, the term vomitoxin may be used in lieu of the term DON in the certification statements.

a. Qualitative Service.

For qualitative service, certify results as being equal to or less than a threshold (e.g., 2 ppm) or as exceeding the threshold.

"DON exceeds 2 ppm."

"DON equal to or less than 2 ppm."

b. Quantitative Service.

Use one of the applicable statements for certifying DON on a quantitative basis.

(1) When DON results are less than or equal to 0.5 ppm:

"DON equal to or less than 0.5 ppm."

(2) Certify DON test results between 0.6 ppm and the conformance limit (e.g., 5 ppm) to the nearest whole number in ppm.

"DON (result rounded to the nearest whole number) ppm."

NOTE: The use of this statement is limited when testing with kits having a conformance limit ending in .5 ppm.

(3) When quantitative test results are equal to the conformance limit (e.g., 2.5 ppm).

"DON (enter conformance limit) ppm."

NOTE: The use of this statement is used only for test kits having a conformance limit ending in .5 ppm.

(4) When quantitative test results are greater than the conformance limit (e.g., 2.5 ppm).

"DON exceeds (enter conformance limit) ppm."

(5) Board Appeals performed by the High Performance Liquid Chromatography (HPLC) method are certified to the tenth ppm.

"DON (record actual results to the nearest tenth) ppm. Results based on HPLC Reference Method."

c. Optional Quantitative Statements.

(1) At the request of the applicant, certify quantitative test results between 0.6 ppm and the conformance limit to the tenth ppm.

"DON (result in tenths) ppm."

(2) At the request of the applicant, use the following statement when DON is not detected using a quantitative method (0.0 ppm).

"DON not detected."

NOTE: If sublot results are combined and averaged and the lot average is equal to 0.0 ppm, but an individual sublot result exceeds 0.0 ppm, then the statement "DON less than or equal to 0.5 ppm" must be used.

d. Additional Statements.

The statements listed below may be used in addition to the applicable qualitative/quantitative statements.

(1) At the request of the applicant, convert and certify the ppm result to parts per billion (ppb) using an approved statement. To convert ppm to ppb, multiply the ppm result by 1000.

"(Actual ppm result) ppm is equivalent to (converted ppb results) ppb."

(2) At the request of the applicant, convert and certify results in milligrams per kilogram (mg/kg) or micrograms per kilogram (µg/kg). Use the following equivalents to determine mg/Kg or µg/kg:

ppm = mg/kg ppb = µg/kg

(3) When certifying multiple DON results on the same certificate and the results are based on different sample types the certificate must reflect the difference. As a guideline, the multiple results are shown as follows:

"Sublot sample results: DON equal to or less than (threshold) ppm."

"Composite sample result: DON (actual result) ppm."

(4) Use this statement when the applicant requests the type of test shown on the certificate:

"Results based on (indicate type of test used) method."

(5) Upon request of the applicant, one of the following statements may precede the applicable results statement when test results are equal to or less than the specified threshold.

"The DON result is negative." OR "Negative DON."

NOTE: These certification statements may be modified as deemed necessary.

e. Reinspection, Appeal, Board Appeal Certificates.

(1) Results are reported on the same kind of certificate issued for the original service and supersede the previously issued inspection certificate.

Enter the following statement on the reinspection/appeal/board appeal certificate:

"This certificate supersedes Certificate No. (number) dated (date)."

(2) The superseded certificate is null and void as of the date of the subsequent (reinspection/appeal/board appeal) certificate.

"The superseded certificate has not been surrendered."

(3) When a file sample is used, enter the following statement on the reinspection/appeal/board appeal certificate:

"Results based on file sample."

(4) When reporting more than one official result on the same certificate but at different levels of inspection, explain this condition using one of the following applicable statements:

"(Grade, factor, or official criteria) results based on (new/file) sample. All other results are those of the original inspection service."

"(Grade, factor, or official criteria) results based on the appeal inspection. All other results are those of the (original inspection/reinspection) service."

"(Grade, factor, or official criteria) results based on the Board appeal inspection. All other results are those of the (original inspection/ reinspection/appeal inspection) service."

1.1 PURPOSE

This directive establishes official procedures for determining Deoxynivalenol (DON) in grain and certifying the official results. This service is provided as official criteria under the authority of the United States Grain Standards Act (USGSA), as amended.

1.2 BACKGROUND

DON, also referred to as vomitoxin, is a naturally occurring mycotoxin produced by several species of Fusarium. Wet and cool weather from flowering time on to maturity promotes infection, resulting in scab or head blight in barley, wheat, oats, and rye.

The Federal Grain Inspection Service (FGIS) of the Grain Inspection, Packers and Stockyards Administration (GIPSA) provides DON testing service as official criteria for wheat, barley, oats, and corn. All official DON testing of grain is performed as prescribed in this directive by authorized employees of FGIS or licensed delegated/designated agency personnel.

Individuals wanting grains officially tested for DON should contact the nearest FGIS field office or delegated/designated agency.

DON test results are not reported to the Food and Drug Administration (FDA) because action limits are not established at this time.

1.3 DISCLAIMER CLAUSE

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

1.4 APPROVED TEST METHODS

The methods listed below have been conformance tested to perform within FGIS specifications. Each of the approved test methods has been certified to provide results accurate up to the conformance test level at which they were approved. Any test results that are above the conformance limits are reported as exceeding the established limit unless a supplemental analysis is performed.

FGIS APPROVED TEST METHODS			
Method and Test Kit	Approved for		Conformance Limit(s)
	Qualitative	Quantitative	
Myco✓ DON (Strategic Diagnostics Inc.)		X	3 PPM
RIDASCREEN®FAST DON (r-Biopharm)		X	5 PPM
AgriScreen (Neogen)	X	X	5 PPM (quantitative test)
Veratox (Neogen)	X	X	5 PPM (quantitative test)
Fluoroquant (Romer)		X	5 PPM
AccuTox™ (Romer)		X	5 PPM
EZ-Quant DON (Diagnostix)		X	5 PPM*
EZ-Quant DON 0.5 ppm (Diagnostix)		X	2.5 PPM*
Veratox 5/5 (Neogen)		X	5 PPM**
DON FQ (Vicam)		X	5 PPM

* The EZ-Quant DON Test kit (part number 600312) for barley, malted barley, wheat, and corn is approved to a conformance limit of 5 ppm. The EZ-Quant DON 0.5 ppm test kit (part number 600313) is exclusively for barley and malted barley and is approved to a conformance limit of 2.5 PPM.

** The Veratox 5/5 test kit is approved to a conformance limit of 5 ppm. Using the optional extraction procedure limits the conformance level to 2.5 ppm.

The following chart lists the DON field test kits and the grains/commodities for which they have been approved. For information concerning the testing of other grains/commodities, contact the Policies and Procedures Branch.

Test Method	Grain/Commodity						
	Barley	Malted Barley	Corn	Oats	Wheat	Wheat Flour	Wheat Midds
DON Fluoroquant	X	X	X	X	X		
DON AccuTox	X	X	X	X	X	X	X
Veratox	X	X	X	X	X		
Veratox 5/5	X	X	X	X	X		
AgriScreen	X	X	X	X	X		
EZ-Quant DON (0.5 -5.0)	X	X	X		X		
EZ-Quant DON (0.5-2.5)	X	X					
RIDASCREEN® FAST DON	X	X	X	X	X		
Myco✔DON	X	X	X	X	X		
DON FQ	X	X	X	X	X		

1.5 TESTING SERVICES

Applicants requesting DON testing must specify whether qualitative or quantitative testing service is desired. If qualitative analysis is requested, the applicant must specify the level desired (e.g., 1, 2 ppm). Three types of DON testing services are available as follows:

a. Submitted Sample Service.

Analysis based on a sample submitted by the applicant for service.

b. Official Sample-Lot Service.

Analysis based on an official sample obtained and analyzed by official personnel.

(1) Single lot inspection.

Samples may be obtained and tested on either an individual carrier basis or a composite sample basis (maximum of five railcars or fifteen trucks per composite sample).

(2) Unit train inspection under the CuSum Loading Plan.

Unit trains are analyzed on a sublot basis for wheat and barley and on a composite basis for other grains. Acceptable sublots must conform to contract specifications when "maximum" limits are specified.

For unit trains, the sublot size for DON testing and for grade analysis may be different. For example, an applicant may request grade analysis on the basis of a sublot containing two cars and request DON analysis on the basis of five cars.

The maximum size sublot for DON testing is five railcars for unit trains consisting of less than 200,000 bushels, or less than 50 cars. For unit trains consisting of 200,00 bushels or more, or 50 railcars or more, the maximum sublot size is ten railcars.

(3) Export shiplots

Export shiplots are analyzed on a sublot basis for wheat and barley and on a composite basis for other grains. Acceptable sublots must conform to contract specifications when "maximum" limits are specified.

The testing frequency for shiplot grain will be the same as the sample for grade analysis unless the applicant specifically requests DON analysis on the basis of a component sample.

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CHAPTER 6

NEOGEN - VERATOX 5/5 DON TEST KIT

6.1 TESTING AREA

The extraction solution and other materials used in the Veratox 5/5 test kit does not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this handbook to ensure a safe and efficient work environment.

6.2 EXTRACTION PROCEDURES

a. Standard Extraction Procedure - Testing Wheat, Oats, Barley, Malted Barley and Corn Tested at the 5 ppm Conformance Limit

(1) Place a sheet of filter paper (Whatman #1 folded or S&S 24-cm pleated or equivalent) into a clean funnel mounted over a 25 x 200 mm (diameter x length) test tube or collection beaker.

(2) Label the collection container with the sample identification.

(3) Thoroughly mix the ground sample and weigh a 50-gram portion.

(4) Place the ground 50-gram portion into an 18-ounce Nasco Whirlpack bag or similar type of sealable plastic bag.

(5) Add 250 ml of distilled or deionized water and shake (by hand or mechanically) for 3 minutes.

(6) Let the extract sit for 2 minutes to enable some of the sample to settle before filtering the extract.

(7) Filter the extract by pouring through the filter paper into the labeled sample jar. Collect a minimum of 15 ml of the extract.

OR

Use a filtering syringe and push 1 - 2 ml of the extract through the syringe and collect the filtrate in a cuvette.

(8) Dilute the sample extract 1:2 (1+1) with deionized or distilled water. (For example, add 1.0 ml of extract to 1.0 ml of deionized or distilled water).

(9) Mix well.

(10) Proceed to test analysis steps.

b. Optional Procedure - Testing Wheat, Oats, Barley, Malted Barley, and Corn at Lower Concentration Levels (between 0.5 - 2.5 ppm).

NOTE: Using the optional extraction method limits the testing range from 0.5 ppm to 2.5 ppm. Any test result above 2.5 ppm is reported as > 2.5 ppm unless a supplemental analysis is performed.

(1) Place a sheet of filter paper (Whatman #1 folded or S&S 24-cm pleated or equivalent) into a clean funnel mounted over a 25 x 200 mm (diameter x length) test tube or collection beaker.

(2) Label the collection container with the sample identification.

(3) Thoroughly mix the ground sample and weigh a 50-gram portion.

(4) Place the ground 50-gram portion into an 18-ounce Nasco Whirlpack bag or similar type of sealable plastic bag.

(5) Add 250 ml of distilled or deionized water and shake (by hand or mechanically) for 3 minutes.

(6) Let material stand for 2 minutes to enable some of the sample to settle before filtering the extract.

(7) Filter the extract by pouring through the filter paper into the labeled sample jar. Collect a minimum of 15 ml of the extract.

OR

Use a filtering syringe and push 1 - 2 ml of the extract through the syringe and collect the filtrate in a cuvette.

(8) Proceed to the analysis steps.

6.3 TEST PROCEDURES

a. Analysis Procedure.

(1) Allow reagents, antibody coated wells, mixing wells, and sample extracts to reach room temperature prior to running the test (approximately one hour).

(2) Remove one red-marked mixing well for each sample to be tested, plus five red-marked wells to be used for controls. Place these wells in the microwell holder.

NOTE: The maximum number of test samples that can be run at one time is 19. Using two strips of 12 wells, designate 5 wells for the controls and the remainder of the wells for test samples.

(3) Remove an equal number of antibody-coated wells. Immediately return antibody wells that will not be used to the foil pack with desiccant. Fold down ends of the pack and seal with tape to protect the antibody. Mark one end of the strip so that the wells can be identified after washing.

(4) Mix each reagent by swirling the reagent bottle prior to use.

(5) Using a new pipette tip for each, transfer 100 µl of conjugate from the blue-labeled bottle into each mixing well.

(6) Using a new pipette tip for each, transfer 100 µl of control and sample extract into the mixing wells as shown below:

Well #	1	2	3	4	5	6	7	8	9	10	11	12
Sample	C 0	C .25	C .5	C 1.0	C 3.0	S1	S2	S3	S4	S5	S6	S7

Where C 0 is the zero control, C .25 is the .25 ppm control, C .5 is the 0.5 ppm control, C 1.0 is the 1.0 ppm control, and C 3.0 is the 3.0 ppm control. S1 is sample 1, S2 is sample 2, etc.

(7) Using a 12- channel pipettor, mix the wells by pipetting the liquid up and down in the tips 3-4 times. Transfer 100 µl to the antibody wells and mix by sliding the microwell holder back and forth on a flat surface for 10 –20 seconds without splashing reagents from the wells. Incubate for **5 minutes** at room temperature (64 –86º F). Discard the red- mixing wells.

(8) Dump the contents of the antibody wells. With a wash bottle or a running stream, fill each antibody well with deionized or distilled water and then dump the water out. Repeat this step 5 times, then turn the wells upside down and tap out on a paper towel until the remaining water has been removed.

(9) Pour the needed volume of substrate from the green-labeled bottle into the green-labeled reagent boat, and with new tips on the 12-channel pipettor, prime and pipette 100 µl of substrate into the wells and mix by sliding back and forth on a flat surface for 10-20 seconds. Incubate for **5 minutes.** Discard the remaining substrate and rinse the reagent boat with water.

(10) Pour the Red Stop solution from the red-labeled bottle (same volume as prepared for substrate) into the red-label reagent boat. Eject the excess substrate from the 12-channel pipettor, prime the tips, and pipette 100 µl of the Red Stop to each well. Mix by sliding back and forth on a flat surface. Discard the tips.

(11) Wipe bottom of microwells with a dry cloth or towel and read in the Awareness Stat-Fax Model 321 PLUS Reader using a 650 nm filter. Air bubbles should be eliminated, as they could affect analytical results. Results should be read within 20 minutes of completion of the test.

b. <u>Reading the Results with the Stat-Fax Model 321 PLUS Microwell Reader.</u>

To begin from the "Ready" prompt, press Menu, key in the test number, and then press Enter. For DON, the Veratox test number is 5.

(1) The screen will read, "Set carrier to A, press enter." Place the wells all the way to the right in the carrier. Push the carrier all the way to the left to line up the notch with the wells, then press enter. The carrier will advance into the reader, and it should start to print.

(2) When the reader is finished reading the strip, the screen will read, "Plot Curve Y/N?"

Press "Yes" (1/A) to print the graph,

Press "No" (0) to skip this feature.

(3) The screen will read, "Accept Curve Y/N ?"

Press "Yes" (1/A) to accept the curve and proceed to read another strip. When finished reading the second strip, press "Clear" twice and the results strip will print, "Test Ended."

Press "No" (0) to end the test.

(4) If a diluted sample extract (see Standard Extraction Procedure) is being analyzed, the reader value for the extract will need to be modified to adjust for the dilution of the extract. If the original extract was diluted 1+1 with water (this is an actual 1:2 dilution), the sample results are multiplied by 2. If the original extract was diluted 1+3 with water (this is an actual 1:4 dilution), the sample results are multiplied by 4.

(5) If the Optional Extraction Procedure was used for testing samples at lower concentration levels (0.5 - 2.5 ppm) samples, the reader values do not need to be adjusted for dilution of the extract because an undiluted extract was analyzed.

NOTE: If the correlation coefficient is less than 0.98 or if the slope exceeds –2.0 (± 0.5), the reader will print, "Invalid Calibration" and no results will be reported. If the slope value consistently reads outside these tolerances, contact Neogen as soon as possible to report these findings.

6.4 REPORTING AND CERTIFYING TEST RESULTS

a. Testing Wheat, Oats, Barley, Malted Barley and Corn Tested at the 5 ppm Conformance Limit using the Standard Extraction Procedure

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

b. Testing Wheat, Oats, Barley, Malted Barley and Corn Tested at the 2.5 ppm Conformance Limit using the Optional Extraction Procedure

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 2.5 ppm. Results exceeding 2.5 ppm are reported as > 2.5 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 2.4 ppm are certified to the nearest whole ppm.

Test results that are equal to the conformance limit (2.5 ppm) are certified as being equal to 2.5 ppm.

Test results over 2.5 ppm are certified as exceeding 2.5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

6.5 SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** (5 ppm for the normal procedure and 2.5 ppm for the optional procedure) is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON value at 9.0 ppm and the conformance limit value is 5 ppm, in order to obtain a true value, dilute 5 ml of the original extract with 10 ml of the extraction solution (distilled/deionized water). The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 3.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

The calculation is as follows:

$$\text{True DON Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result}$$

In this example: True DON Value = (15 ÷ 5) x 3.1 ppm
= 3 x 3.1 ppm = 9.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the conformance limit of the test kit.

6.6 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

6.7 WASTE DISPOSAL

After the test has been completed, the remaining sample extract and sample solutions may be poured down the drain. Discard solid material in the trash can for routine disposal.

6.8 EQUIPMENT AND SUPPLIES

a. Materials Provided in Test Kits:

(1) 48 Monoclonal-Coated microwells.

(2) 48 Red-Marked Mixing Wells.

(3) 5 Yellow labeled Bottles - 1.5 ml each of 0, 0.25, 0.5, 1.0, and 3.0 ppm DON Controls.

(4) 1 Blue-Labeled Bottle - DON-HRP Conjugate Solution.

(5) 1 Green-Labeled Bottle - 24 ml K-Blue Substrate Solution.

(6) 1 Red-Labeled Bottle - 32 ml Red Stop Solution.

b. Materials Required but not Provided:

(1) Extraction Materials: Whirlpack Bags -18 oz., or equivalent.

(2) Microwell Strip Reader: Awareness Technology STAT-FAX Model 321 PLUS Reader with a 650 nm filter.

(3) 12-Channel multi-channel pipettor and pipette tips.

(4) 100 µl pipettor and pipette tips.

(5) 100 ml pipettor and pipette tips.

(6) Deionized or Distilled Water.

(7) Absorbent Materials: Paper Towels, Kay Dry paper or equivalent.

(8) Waste receptacle.

(9) Microwell holder.

(10) Timer: 3-channel minimum.

(11) Waterproof marker: Sharpie or equivalent.

(12) Wash Bottle.

(13) Reagent Boats.

(14) Sample grinder.

(15) Balance.

(16) Neogen Filter Syringe.

6.9 STORAGE CONDITIONS

Test kits should be refrigerated at temperatures between 36º F and 46º F.

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CHAPTER 10

r-BIOPHARM RIDASCREEN®FAST DON TEST KIT

10.1 TESTING AREA

The extraction solution and other materials used in the r-Biopharm RIDASCREEN®FAST DON test kit does not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in the handbook to ensure a safe and efficient work environment.

10.2 EXTRACTION PROCEDURES

a. Place 50 grams of ground sample into a suitable container (e.g., plastic bag).

b. Add 250 ml of distilled/deionized water and seal/close container securely to prevent spillage.

c. Shake vigorously (by hand or mechanically) for three minutes.

d. Let the extract sit for 2-3 minutes to allow for some settling of the slurry.

e. Filter the extract through Whatman #1 filters (or equivalent) into a clean container that is labeled with sample ID number.

f. Dilute the filtered extract with one part sample extract to 3 parts distilled/deionized water. (e.g., 1 ml sample extract plus 3 ml water)

g. Use 50 µl of the diluted filtrate per well in the test.

10.3 PREPARATION OF SOLUTIONS

a. To prepare the Wash Solution, dissolve the contents of the packet containing the buffer salt in 1 liter of distilled water.

b. Swirl to mix.

When stored properly (at 39º F) the solution has a shelf life of four weeks.

10.4 TEST PROCEDURES

a. Analysis Procedure.

(1) Allow reagents and antibody wells to reach room temperature (68 - 77º F) prior to running the test.

(2) Insert a sufficient number and wells into the microwell holder for all standards and samples to be tested. (For example: to test 11 samples use 16 wells - 5 for the standards and 11 for the test samples).

Test Strip #1

Well #	1	2	3	4	5	6	7	8
Sample	C 0	C .222	C .666	C 2.0	C 6.0	S1	S2	S3

Test Strip #2

Well #	1	2	3	4	5	6	7	8
Sample	S4	S5	S6	S7	S8	S9	S10	S11

Where C 0 is the zero control, C .222 is the 0.222 ppm control, C .666 is the 0.666 ppm control, C 2.0 is the 2.0 ppm control, and C6 is the 6.0 ppm control. S1 is sample 1, S2 is sample 2, S3 is sample 3, etc.

NOTE: Do not run more than 3 strips (19 samples) per set of control standards.

(3) Using a new pipette tip for each standard and sample, pipet 50 µl of standards and prepared sample to separate wells.

(4) Add 50 µl of enzyme conjugate (red capped bottle) into each well.

(5) Add 50 µl of deoxynivalenol antibody (black capped bottle) into each well.

(6) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(7) Incubate for 5 minutes (± 1 minute) at room temperature.

(8) Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.

(9) Using a wash bottle, fill each well with washing buffer solution. Empty the wells again and remove all remaining liquid. Repeat this step 2 times (total of 3 washes).

(10) Add 100 µl of substrate/chromagen (white dropper bottle) to each well.

(11) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(12) Incubate for 3 minutes (± 0.5 minutes) at room temperature (64 – 86º F). Cover the wells with a paper towel to protect them from light sources.

(13) Add 100 µl of stop solution (yellow or orange dropper bottle) to each well.

(14) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(15) Measure absorbance at 450 nm using the Biotek EL 301, Awareness Technology Stat-Fax Model 303 PLUS, or the Hyperion Microreader™ 3 Model 4027-002, microwell readers.

(Results must be read within 10 minutes)

b. Reading the Results.

(1) Biotek EL 301 Microwell Reader.

(a) Make sure that the microwell reader is on and allowed to warm-up for a minimum of 15 minutes before using.

(b) Remove sample carriage and hit "Enter."

(c) Insert W2 filter and hit "Enter."

(d) Insert W1 filter (450 nm) and hit "Enter."

(e) Hit "Clear" and then "Blank." This will cause the instrument to read air as the blank sample.

(f) Load antibody-coated wells into sample carriage so that the first control labeled 0 is in position A1.

(g) Load the sample carriage into the strip reader so that position A1 is under the light beam of the reader.

(h) Press "Read" and an absorbance value for A1 should appear in the display on the microwell reader. Record the value.

(i) Slide the carriage to position A2 and press "Read." An absorbance value for A2 will appear. Record the value.

(j) Repeat step (i) until absorbance values have been obtained for all controls and samples. Record the values.

(k) Use the RIDA®SOFT Win Data software provided by r-Biopharm to convert the absorbance values into concentration values.

(2) Stat-Fax Model 303 PLUS Microwell Reader

(a) To begin from the "Ready" prompt, press Menu, key in the test number, and then press Enter.

(b) The screen will read, "Set carrier to A, press enter." Place the wells all the way to the right in the carrier. Push the carrier all the way to the left to line up the notch with the wells, then press enter. The carrier will advance into the reader, and it should start to print.

(c) When the reader is finished reading the strip, the screen will read, "Plot Curve Y/N?"

Press "Yes" (1/A) to print the graph,

Press "No" (0) to skip this feature.

(d) The screen will read, "Accept Curve Y/N ?"

Press "Yes" (1/A) to accept the curve and proceed to read another strip. When finished reading the second strip, press "Clear" twice and the results strip will print, "Test Ended."

Press "No" (0) to end the test.

(3) Hyperion Microreader™ 3 Model 4027-002 Microwell Reader

(a) After the power is turned on the instrument will proceed through a calibration mode then advance to the "Main Menu" setting.

(b) When prompted to "Run a test", select yes, select the appropriate test number, then press "Enter".

(c) At the "Run XXX test?" prompt select yes, select the number of wells (e.g., 8, 12, 16, 24) then press "Enter".

(d) At the "Insert strip" prompt insert the test well strip and press "Y" to continue.

(e) The reader will read the optical density of the wells and print a report.

(f) After the report is printed a "Continue test" prompt will appear. To continue testing select yes and follow the to the instrument prompts as indicated above.

(g) Use the RIDA®SOFT Win Data software provided by r-Biopharm to convert the absorbance values into concentration values.

10.5 REPORTING AND CERTIFYING TEST RESULTS

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

10.6 SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON value at 9.0 ppm and the conformance limit value is 5 ppm, in order to obtain a true value, dilute 5 ml of the original extract with 10 ml of the extraction solution (distilled/deionized water). The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 3.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

The calculation is as follows:

$$\text{True DON Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result}$$

In this example: True DON Value = (15 ÷ 5) x 3.1 ppm
= 3 x 3.1 ppm = 9.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the conformance limit of the test kit.

10.7 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

10.8 WASTE DISPOSAL

After the test has been completed, the remaining sample extract and sample solutions may be poured down the drain. Discard solid material in the trash can for routine disposal.

10.9 EQUIPMENT AND SUPPLIES

a. Materials Provided in Test Kits (48 well kit).

(1) 1 microtiter plate.

(2) 48 Antibody coated wells.

(3) 5 DON standard solutions of 1.3 ml each; 0, 0.222, 0.666, 2.0, and 6.0 ppm DON in water.

(4) 1 red-capped bottle of 3 ml peroxidase conjugated deoxynivalenol solution.

(5) 1 black-capped bottle of 3 ml anti- deoxynivalenol antibody.

(6) 1 white dropper bottle of 6 ml Substrate/Chromagen, stained red.

(7) 1 yellow or orange dropper bottle of Stop reagent.

(8) 1 packet of washing buffer (salt).

b. Materials Required but not Provided.

(1) Biotek EL 301 Microwell Reader, Awareness Technology Inc. Stat-Fax Model 303 PLUS, or Hyperion MicroReader™ 3 Model No. 4027-002 with 450-nm filters.

(2) RIDA™SOFT Win Software.

(3) 50 µl, 100 µl, and 1000 µl Pipettor and pipette tips.

(4) Graduated cylinders (plastic or glass): 100 ml, 1 liter.

(5) Sample shaker (optional).

(6) Filter funnel.

(7) Whatman #1 filter paper or equivalent.

(8) Balance.

(9) Stepper pipetter.

(10) Paper towels, Kaydry paper or equivalent absorbent material.

(11) Waste receptacle.

(12) Timer: 3 channel minimum.

(13) Waterproof marker, Sharpie or equivalent.

(14) Wash bottle.

(15) Deionized or distilled water.

10.10 STORAGE CONDITIONS

The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 36° F and 46° F.

When stored properly (at 39º F) the Wash Solution has a shelf life of four weeks.

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CHAPTER 11

STRATEGIC DIAGNOSTICS INC. - MYCO✔ DON TEST KIT

11.1 TESTING AREA

The extraction solution and other materials used in the Myco✓ DON test kit does not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this handbook to ensure a safe and efficient work environment.

11.2 EXTRACTION PROCEDURES

a. Weigh 50 grams of ground sample and place in a clean blender container with a tight fitting lid.

b. Add 250 ml of distilled or deionized water.

c. Blend at high speed for 2 minutes.

d. Allow the extract to stand for 2-3 minutes to allow the sample slurry to settle.

e. Filter a minimum of 15 ml of the extract through a Whatman #1 filter and collect the extract in a clean container that is labeled with the sample ID number.

f. Proceed to the test procedures.

11.3 PREPARATION OF SOLUTIONS

a. To prepare the Wash Solution, transfer the contents of the Wash Concentrate vial to a 500 ml plastic squeeze bottle and add 475 ml of distilled or deionized water.

b. Swirl to mix.

11.4 TEST PROCEDURES

a. Analysis Procedure.

(1) Allow reagents, antibody coated wells, mixing wells, and sample extracts to reach room temperature prior to running the test (approximately one hour).

(2) Place the appropriate number of red mixing wells and clear test wells into a microwell holder.

(3) Dispense 100 µl of enzyme conjugate into each well using a pipette.

(4) Using a clean pipette tip for each transfer, dispense 100 µl of each calibrator and sample into the appropriate mixing wells using an adjustable or fixed 100 µl pipette.

Well #	1	2	3	4	5	6	7	8	9	10	11	12
Sample	C 0	C .25	C .5	C 1	C 3	S1	S2	S3	S4	S5	S6	S7

Where C 0 is the zero control, C .25 is the 0.25 ppm control, C .5 is the 0.5 ppm control, C 1 is the 1.0 ppm control, and C3 is the 3.0 ppm control. S1 is sample 1, S2 is sample 2, etc.

(5) Using a multi-channel pipette, mix the contents of the wells by repeatedly filling and emptying the tips into the mixing wells.

(6) Using a multi-channel pipette, transfer 100 µl of each reaction mixture directly into all corresponding clear test wells. Discard the mixing wells into an appropriate waste container.

(7) Let the reaction mixture incubate for exactly 5 minutes. Mix the solution in the wells by gently swirling the plate on a flat surface for the first 15 seconds.

(8) At the end of the 5 minute incubation period, dump the contents of the wells into an appropriate waste container. Using a 500 ml squeeze bottle containing the wash solution, vigorously wash each well by overfilling. Repeat the vigorous wash for a total of four washes. After the last wash, invert the wells and tap on absorbent paper to remove the residual wash solution. Wipe excess liquid from the bottom of the wells.

(9) Pour the Substrate Solution into a clean reagent reservoir.

(10) Dispense 100 µl of Substrate Solution into each test well using a multi-channel pipette.

(11) Let the Substrate Solution incubate for exactly 5 minutes. Mix the solution in the wells by gently swirling the plate on a flat surface for the first 15 seconds.

(12) Pour the Stop Solution into a clean reagent reservoir.

(13) Dispense 100 µl of Stop Solution into each test well using a multi-channel pipette.

(14) Within 20 minutes, read and record the optical density at 650 nm using a Hyperion MicroReader™ 3 Model 4027-002 microwell reader. Make sure that the well bottoms are clean and dry before placing in reader.

b. Reading the Results.

(1) After the power is turned on the instrument will proceed through a calibration mode then advance to the "Main Menu" setting.

(2) When prompted to "Run a test", select yes, select the appropriate test number, then press "Enter".

(3) At the "Run XXX test?" prompt select yes, select the number of wells (e.g., 8, 12, 16, 24) then press "Enter".

(4) At the "Insert strip" prompt insert the test well strip and press "Y" to continue.

(5) The reader will read the optical density of the wells and print a report.

(6) After the report is printed a "Continue test" prompt will appear. To continue testing select yes and follow the to the instrument prompts as indicated above.

(7) Use the data reduction software provided by SDI to quantify results.

11.5 REPORTING AND CERTIFYING TEST RESULTS

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 3.4 ppm. Results exceeding 3.4 ppm are reported as > 3.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 3.4 ppm are certified to the nearest whole ppm.

Test results over 3.4 ppm are certified as exceeding 3 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

11.6 SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON result at 6.0 ppm and the conformance limit value is 3 ppm, in order to obtain a true value, dilute 5 ml of the original extract with 10 ml of the extraction solution (distilled/deionized water). The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 2.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 6.3 ppm (3 x 2.1).

The calculation is as follows:

$$\text{True DON Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result}$$

In this example: True DON Value = (15 ÷ 5) x 2.1 ppm
= 3 x 2.1 ppm = 6.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the conformance limit of the test kit.

11.7 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

11.8 WASTE DISPOSAL

After the test has been completed, the remaining sample extract and sample solutions may be poured down the drain. Discard solid material in the trash can for routine disposal.

11.9 EQUIPMENT AND SUPPLIES

a. Materials Provided in Test Kits.

(1) 48 antibody-coated microtiter wells (4 strips of 12) in foil pouch.

(2) 48 red-marked mixing wells in poly bag.

(3) 5 vials each containing 2 ml of: 0, 0.25, 0.5, 1.0, and 3.0 ppm of DON calibration.

(4) 1 vial containing 8 ml of DON-HRP Enzyme Conjugate.

(5) 1 vial containing 8 ml of Substrate.

(6) 1 vial containing 8 ml of Stop Solution.

(7) 1 vial containing 25 ml of 20X Wash Concentrate.

(8) 4 multi-channel pipette reservoirs.

(9) Data reduction software. (Provided separately)

b. Materials Required but not Provided.

(1) Distilled/deionized water.

(2) 100 ml graduated cyclinder.

(3) Glassware with 125 ml capacity, for sample extraction.

(4) Whatman #1 filter paper or equivalent.

(5) Filter funnel.

(6) 100 µl pipette with disposable tips.

(7) Multi-channel pipette.

(8) 500 ml plastic squeeze bottle.

(9) Hyperion MicroReader™ 3 Model 4027-002 with 650 nm filter.

(10) Timer.

(11) Blender and blender jars.

(12) Balance.

(13) Sample Grinder.

11.10 STORAGE CONDITIONS

The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 36° F and 46° F.

DEPARTMENT OF AGRICULTURE
GRAIN INSPECTION, PACKERS AND STOCKYARDS ADMINISTRATION
FEDERAL GRAIN INSPECTION SERVICE
STOP 3630
WASHINGTON, D.C. 20090-3630

DON HANDBOOK
12-23-02

DON (Vomitoxin) Testing

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